Annals of Warsaw University of Life Sciences – SGGW
Animal Science No 54 (1), 2015: 45–49
(Ann. Warsaw Univ. of Life Sci. – SGGW, Anim. Sci. 54 (1), 2015)

# Relationships between milk β-carotene concentrations and the cytological quality of cow's milk

BEATA KUCZYŃSKA, ALEKSANDRA KAPUSTA, KAMILA PUPPEL, TERESA NAŁĘCZ-TARWACKA, ARKADIUSZ BUDZIŃSKI, MARCIN GOŁĘBIEWSKI, MARTA CZUB, HENRYK GRODZKI
Department of Animal Breeding and Production, Warsaw University of Life Sciences – SGGW

**Abstract:** *Relationships between milk β-carotene concentrations and the cytological quality of cow's milk.* The objective of the study was to describe the relationship between milk β-carotene concentration and the cytological quality of milk of high yielding cows. The experiment was carried out at the research dairy farm of the Warsaw University of Life Sciences – SGGW (WULS). From a herd of 320 Polish Holstein--Friesian cows maintained in a free-stall dairy shed 58 cows were selected taking into consideration the stage of lactation (15 ±14 days). Cows were fed a total mixed ration (TMR) diet provided *ad libitum*. Four groups of cows taking into consideration concentration of β-carotene in milk has been created: (1) below 150 mg/l; (2) 151–250 mg/l; (3) 251–450 mg/l; (4) beyond 450 mg/l. The content of β-carotene and SCC amounted at the herd level: 192 $10^3$ ml and 0.312 mg/l respectively. The β-carotene content ranged from 0.150 to 0.451 mg/l. The highest content of β-carotene, 0.451 mg/l, was found in milk of cows with the lowest level of somatic cells count (SCC). The lowest level, 0.150 mg/l, was found in milk of cows with the highest level of SCC. Milk SCC varies significantly with the concentration of β-carotene in cow's milk. Researchers should consider monitoring for this variable as potential cofounder when exploring the relationship between *mastitis*, intramammary infection and nutritional management.

*Key words*: β-carotene, somatic cells count, immunity

## INTRODUCTION

*Mastitis* is the most common infectious disease of dairy cows and results in economic loss for both dairy farmers and for dairy's (Geary et al. 2012). The production of high quality milk is a requirement to sustain a profitable dairy industry and somatic cell count (SCC) values are routinely used to identify subclinical *mastitis* (Philpot and Nickerson 2000). Age, number of quarters with intramammary infection, season, nutrition are significant factors that have been associated with SCC (Schepers et al. 1997, Kuczyńska 2011, Puppel et al. 2012). Increased SCC with parity may be attributed to increased prevalence of intramammary infection and greater cellular response to certain pathogens.

β-carotene stabilizes the free radicals, as meaning breaking the sequence of radical reactions, and prevents radical degradation processes of cell components (Kankofer and Albera 2007). Carotenoids have diverse biological functions and actins, the most important of which are effective antioxidants *in vitro*, but the situation *in vivo* is less clear. It stabilizes the free radicals, as meaning breaking the sequence of radical reactions, and prevents radical degradation processes of cell components. Preferably also operates on the reproductive system. It has been proved that the higher content of β-carotene reduces the incidence of retained placenta, and accelerates and supports ovarian luteal activity (Kankofer and Albera 2007, Kawashima et al. 2009, Kumar et al. 2010). Carotenoids have also been reported to have immunomodulatory effects, such as the reduction in UV-induced immunosuppresion and the increase in natural killer cell activity after dietary supplementation with β-carotene (Van den Berg et al. 2000).

Low plasma concentrations of β-carotene is associated with increased incidence of udder infection. Carotenoids also increase the lymphocytes population and their activity, stimulate phagocytosis (Chew 1993, Michal et al. 1994). The carotenoid content in roughage largely depends on environmental conditions. Important is the method of harvest, weather conditions, as well as a method of preserving feed (Bergamo 2003, Krzyżewski 2012). It has been proved that the drying may result in loss of β-carotene of as much as 83%. Ensiling process results in less losses but they get deeper as time goes on storage silage in a silo or prism (Krzyżewski 2012).

The objective of the study was to describe the relationship between milk β--carotene concentration and the cytological quality of milk of high yielding cows.

## MATERIAL AND METHODS

The experiment was carried out at the research dairy farm of the Warsaw University of Life Sciences – SGGW (WULS). From a herd of 320 Polish Holstein-Friesian cows maintained in a free-stall dairy shed 58 cows were selected taking into consideration the stage of lactation (15 ±14 days). Cows were fed a total mixed ration (TMR) diet provided *ad libitum*, formulated using the INRA system (Table 1).

TABLE 1. Ingredient composition of the TMR diet

| Ingredients | TMR diet |
|---|---|
| Feeding ration (kg) | |
| Maize silage | 23.33 |
| Alfaalfa silage | 10.83 |
| Maize corn silage | 3.70 |
| Concentrate | 5.12 |
| Straw | 1.00 |
| Total | 43.98 |
| Concentrate (kg) | |
| Hydropalm – by pass oil | 0.60 |
| Fodder chalk | 0.15 |
| $NaHCO_3$ | 0.12 |
| BetaLac – premix | 0.13 |
| NaCl | 0.05 |
| Rapeseed meal | 2.23 |
| Soya meal | 2.30 |
| Grain meal | 1.30 |
| Rumex | 0.005 |
| $Ca_3(PO4)_2$ | 0.05 |
| Total | 6.94 |

### Sampling

Representative milk samples were collected from each cow during milking by means of a milk meter in the milking parlor. Milk samples were taken individually from each cow 10 times during the experiment at monthly intervals.

### Milk analyses

Analysis of the somatic cell count (SCC) was performed using a Somacount-150 counter (Bentley Poland, Warsaw, Poland).

Analysis of β-carotene was established using an Agilent 1100 Series reverse phase high-performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) and Zorbax Eclipse XDB C8 column (4.6 × 150 mm, 5 µm film thickness) according to the method described by Puppel et al. (2012).

Four groups of cows taking into consideration concentration of β-carotene in milk has been created: (1) below 150 mg/l; (2) 151–250 mg/l; (3) 251–450 mg/l; (4) beyond 450 mg/l.

**Statistical analyses**

The data obtained were analyzed statistically by multifactor analysis of variance using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

$$Y_{ijkl} = \mu + A_i + B_j + (A_i \times B_j) + e_{ijk}$$

where:

$Y_{ijkl}$ – dependent variable;
$\mu$ – general mean;
$A_i$ – β-carotene effect ($i$ = 1–4, where: (1) < 150 mg/l; (2) 151–250 mg/l; (3) 251–450 mg/l; (4) > 450 mg/l);
$B_j$ – SCC effect;
$A_i \times B_j$ – interaction between β-carotene content and SCC;
$e_{iij}$ – standard error.

RESULTS AND DISCUSSION

The content of β-carotene and somatic cells count (SCC) amounted at the herd level: 192 $10^3$/ml and 0.312 mg/l respectively (Table 2). The overall mean for β-carotene content were similar to the value obtained by Kuczyńska (2011).

TABLE 2. β-carotene and somatic cells count of bovine milk during experiment

| Ingredient | N | LSM | SEM |
|---|---|---|---|
| Somatic cells count ($10^3$/ml) | 580 | 192$^A$ | 26.674 |
| β-carotene (mg/l) | 580 | 0.312$^A$ | 0.0083 |

LSM – least square of the mean, SEM – standard error of the mean, values in the column marked with the same letters differ significantly at $P \leq 0.01$.

In contrast, Morris (2002) reported lower concentration of β-carotene (0.1 mg/l). Milk composition can be altered by the feeding regime. The content of β-carotene was shown to be transferred either directly from the feed to the milk (Kuczyńska 2011) or from supplements added to the cows'diet (Puppel et al. 2012). Puppel et al. (2012) reported, that modification of the diet of cows with fish oil and linseed significantly influenced antioxidant properties of their milk; however, the response of multiparous and primaparous cows was noticeably different to the supplement introduced.

The content of β-carotene ranged at the herd level from 0.150 to 0.451 mg/l. The highest content of β-carotene, 0.451 mg/l, was found in milk of cows with the lowest level of SCC (Table 3).

TABLE 3. Effect of β-carotene concentration in milk on the somatic cells count

| β-carotene (mg/l) | N | Somatic cells count ($10^3$/ml) | SEM |
|---|---|---|---|
| 0.150 | 112 | 279$^{ABC}$ | 60.653 |
| 0.151–0.250 | 186 | 210$^{AD}$ | 47.066 |
| 0.251–0.450 | 164 | 160$^{BDE}$ | 50.124 |
| > 0.451 | 118 | 125$^{CDE}$ | 59.091 |

SEM – standard error of the mean, values in the column marked with the same letters differ significantly at $P \leq 0.01$.

The lowest level, 0.150 mg/l, was found in milk of cows with the highest level of SCC. LeBlanc (2004) reported, that concentration of β-carotene in milk from cows with *mastitis* is almost half lower (0.070 mg/l), than from cows with healthy gland (0.160 mg/l). Cows suffering from severe mastitis tend to produce milk containing less β-carotene and more retinol than non-infected cows (Chew et al. 1993). It has been proved, that the higher content of β-carotene reduces the incidence of retained placenta, and accelerates and supports ovarian luteal activity (Kankofer 2007, Kawashima et al. 2009, Kumar et al. 2010). It can therefore concluded that carotenoids also affect the immunity of the mammary gland, slow down the inflammatory process (*mastitis*), and thus contribute to a lower content of SCC in milk (Chew 1993, Michal 1994).

## CONCLUSION

Milk SCC varies significantly with the concentration of β-carotene in cow's milk. Researchers should consider monitoring for this variable as potential cofounder when exploring the relationship between *mastitis*, intramammary infection and nutritional management.

**Acknowledgement**

Researches was supported by National Science Center and realized within the project NN 311 55 8840 entitled "Relationship between concentration of bioactive substances in milk during standard lactation and blood biochemical parameters of high yielding Polish Holstein-Friesian cow's".

## REFERENCES

BERGAMO P., 2003: Fat soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products. Food Chem. 82 (4): 625–631.

CHEW B.P., 1993: Role of carotenoids in the immune response. J. Dairy Sci. 76: 2804–2811.

GEARY U., LOPEZ-VILLALOBOS N., BEGLEY N., MCCOY F., O'BRIEN B., O'GRADY L., SHALLOO L., 2012: Estimating the effect of mastitis on the profitability of Irish dairy farms. J. Dairy Sci. 95: 3662–3673.

KANKOFER M., ALBERA E., 2007: The concentration of vitamin a and its provitamin – β-carotene in bovine retained and not retained placenta. Acta Vet. (Beograd), 57 (2–3): 181––189.

KAWASHIMA C., NAGASHIMA S., SAWADA K., SCHWEIGERT F.J, MIYAMOTO A., Kida K., 2010: Effect of β-carotene supply during close-up dry period on the onset of first postpartum luteal activity in dairy Cows. Reprod. Dom. Anim. 45: 282–287.

KRZYŻEWSKI J., STRZAŁKOWSKA N., BAGNICKA E., JÓŹWIK A., HORBAŃCZUK J., 2012: Wpływ antyoksydantów zawartych w tłuszczu pasz objętościowych na jakość mleka krów. Żywność – Nauka – Technologia – Jakość 3 (82): 35–45.

KUCZYŃSKA B., 2011: Bioactive components and technological parameters of milk produced at ecological and conventional farms. Dissertations and Monographs. Wydawnictwo SGGW, Warsaw (In Polish, with an English abstract).

KUMAR S., PANDEY A.K., MUTHA M.R., RAZZAQUE W.A.A., 2010: Role of β-carotene / vitamin A in animal reproduction. Vet. World 3 (5): 236–237.

LeBLANC S.J., HERDT T.H., SEYMOUR W.M., DUFFIELD T.F., LESLIE K.E., 2004: Peripartum serum vitamin E, retinol, and beta-carotene in dairy cattle and their associations with disease. J. Dairy Sci. 87: 609–619.

MICHAL J.J., HEIRMAN L.R., WONG T.S., CHEW B.P., 1994: Modulatory, effects of dietary on blood and mammary leukocyte function in periparturient dairy cows. J. Dairy Sci. 77: 1408–1421.

MORRIS C.A., KNIGHT T.W., NEWMANS S.A.N., HICKEY S.M., DEATH A.F., O'NEILL

K.T., RIDLAND M., 2002: Genetic studies of carotenoids concentration in the plasma and milk of New Zeland dairy cattle. New Zeland J. Agri. Res. 45: 27–33.

PHILPOT W.N., NICKERSON S.C., 2000: Winning the fight against mastitis. Westfalia-Surge, Inc.: 13–15.

PUPPEL K., NAŁĘCZ-TARWACKA T., KUCZYŃSKA B., GOŁĘBIEWSKI M., KORDYASZ M., GRODZKI H., 2012: The age of cows as a factor shaping the antioxidant level during a nutritional experiment with fish oil and linseed supplementation for increasing the antioxidant value of milk. J. Sci. Food Agric. 92: 2494–2499.

SCHEPERS A.J., LAM T.J., SCHUKKEN Y.H., WILMINK J.B., HANEKAMP W.J., 1997: Estimation of variance components for somatic cell counts to determine thresholds for uninfected quarters. J. Dairy Sci. 80: 1833–1840.

Van den BERG H., FAULKS R., FERNANDO GRANADO H., HIRSCHBERG J., OLMEDILLA B., SANDMANN G., SOUTHON S., STAHL W., 2000: The potential for the improvement of carotenoid levels in foods and the likely systemic effects. J. Sci. Food Agric. 80: 880–912.

**Streszczenie:** *Zależność między zawartością β-karotenu a jakością cytologiczną mleka.* Celem pracy było określenie zależności między koncentracją β-karotenu a jakością cytologiczną mleka wysokowydajnych krów rasy PHF. Doświadczenie przeprowadzono w Rolniczym Zakładzie Doświadczalnym SGGW Obory – Wilanów. Ze stada podstawowego liczącego 320 krów rasy polskiej holsztyńsko-fryzyjskiej, utrzymywanych w systemie wolnostanowiskowym, wyselekcjonowano 58 krów, biorąc pod uwagę stadium laktacji (15 ±14 dni), jak również i wiek krów (wieloródki). Krowy objęte były całorocznym żywieniem TMR, *ad libitum*. Po uzyskaniu wstępnych wyników, krowy zostały podzielone na cztery grupy, biorąc pod uwagę średnią koncentrację β-karotenu w tłuszczu mlekowym: (1) poniżej 150 mg/l; (2) 151–250 mg/l; (3) 251–450 mg/l; (4) powyżej 450 mg/l. Prezentowane wyniki stanowią średnią z 10 pobrań uzyskanych od krów w trakcie trwania laktacji 305-dniowej. Średnia zawartość β-karotenu i LKS w mleku krów dla całego stada wyniosła odpowiednio 0.312 mg/l i 192 tys./ml. Biorąc pod uwagę zawartość LKS w mleku, koncentracja β-karotenu znajdowała się w przedziale od 0.150 do 0.451 mg/l. Największą zawartość β-karotenu, 0.451 mg/l, wykazano w mleku krów o najwyższej jakości cytologicznej. Najniższy poziom natomiast w mleku o najwyższym poziomie LKS – 279 $10^3$/ml. Badania wykazały, że poziom LKS w istotny sposób kształtowany jest przez stężenie β-karotenu w mleku krów. Dlatego też, powinno się rozważyć monitorowanie tej zmiennej jako potencjalnego czynnika w trakcie określania zależności między zakażeniem wymienia, *mastitis* i bilansowaniem dawek pokarmowych w bioaktywne składniki dla wysokowydajnych krów.

*MS received May 2015*

**Authors' address:**
Beata Kuczyńska
Zakład Hodowli Bydła
Katedra Szczegółowej Hodowli Zwierząt
Wydział Nauk o Zwierzętach SGGW
ul. Ciszewskiego 8, 02-786 Warszawa
Poland
e-mail: beata_kuczyńska@sggw.pl